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BDH2 promotes apoptosis and autophagy of lung adenocarcinoma cells *via* Akt/mTOR pathway

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Abstract. Cytoprotective autophagy induces tumor cell apoptosis or autophagic programmed cell death. Autophagy and apoptosis are implicated in the pathogenesis of lung cancer, especially lung adenocarcinoma. 3-Hydroxybutyrate dehydrogenase type 2 (BDH2), a rate-limiting catalyzer in the regulation of intracellular iron metabolism and siderophore biogenesis, has been shown to be a tumor suppressor through promotion of cell apoptosis and autophagy. However, the biological role of BDH2 on lung adenocarcinoma cell apoptosis and autophagy remains unclear. Data from Western blot and qRT-PCR showed that BDH2 was down-regulated in lung adenocarcinoma cells (A549, NCI-H1975, PC9) compared to normal human lung cells (BEAS-2B). Functional assays demonstrated that pcDNA-mediated over-expression of BDH2 reduced cell viability of lung adenocarcinoma cells, and repressed the proliferation. Cell apoptosis of lung adenocarcinoma was promoted by BDH2 over-expression with up-regulation of Bax and cleaved caspase-3. Over-expression of BDH2 reduced protein expression of p62 in lung adenocarcinoma cells, enhanced LC3 and Beclin-1. Phosphorylation of AKT and mTOR in lung adenocarcinoma cells were reduced by BDH2 over-expression. In conclusion, BDH2 functioned as a tumor suppressor in lung adenocarcinoma through promotion of Akt/mTOR-mediated cell apoptosis and autophagy.

Key words: BDH2 — Lung adenocarcinoma — Akt/mTOR — Proliferation — Apoptosis — Autophagy

Abbreviations: BDH2, 3-hydroxybutyrate dehydrogenase type 2; NSCLC, non-small cell lung cancer.

Introduction

Lung cancer, the leading cause of cancer death, ranks second most common cancer, and the 5-year survival rate of lung cancer is only 18% (Milošević et al. 2016). Small cell lung cancer and non-small cell lung cancer (NSCLC) are the two types of lung cancer, in which NSCLC accounts for almost 85% of all the lung cancer cases (Tian et al. 2020). Although current strategies, including radiotherapy, chemotherapy, surgical resection, immunotherapy and target therapy, have been shown to afford curative treatment for NSCLC, the 10-year survival rate is still less than 10% due to the tumor metastasis, relapse and drug resistance (Barone et al. 2015). Lung adenocarcinoma, the most common type of NSCLC, becomes one of the leading causes of cancer-related mortality worldwide due to the uncontrolled and abnormal lung cell growth (Gardiner et al. 2014). The prognosis of advanced lung adenocarcinoma remains poor (Kudo et al. 2018), and development of novel therapeutic agents beneficial for the treatment of lung adenocarcinoma is urgently needed.

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Autophagy, self-digestion of damaged mitochondria, misfolded proteins or other unwanted components, participates in cell growth and survival and is important for the maintenance of energy homeostasis (Chen et al. 2021). Autophagy might either provide energy for the excessive growth and proliferation of tumor cells or destroy energy homeostasis to promote tumor cell apoptosis and autophagic programmed cell death (Chen et al. 2021). Autophagy has also been shown to exert collaborative, promoting or antagonistic effects on cell apoptosis during the cancer development (Xie et al. 2020). Promotion of autophagy induces autophagic death and apoptosis of lung adenocarcinoma cells (Wan et al. 2020). Therefore, autophagy and apoptosis are regarded as potential therapeutic strategies for the treatment of lung adenocarcinoma (Wu et al. 2021).

3-Hydroxybutyrate dehydrogenase-2 (BDH2) is widely known as dehydrogenase to catalyze siderophore biogenesis, thus implicating in the processes of cell apoptosis, energy metabolism and iron homeostasis (Fu et al. 2021). Increasing evidence has shown that BDH2 suppressed cell proliferation of liver cancer (Liang et al. 2018), and inactivation of BDH2 promoted nasopharyngeal carcinoma cell proliferation and metastasis (Li et al. 2020). Moreover, BDH2 has been shown to inhibit autophagy and promoted mitochondrial apoptosis to repress hepatocellular carcinoma progression (Liang et al. 2019). Reactive oxygen species-induced autophagy was promoted by BDH2 to inhibit gastric cancer growth (Liu et al. 2020). However, the role of BDH2 on lung adenocarcinoma cell apoptosis and autophagy has not been reported yet.

This study assessed expression of BDH2 in lung adenocarcinoma cells, and investigated the biological function of BDH2 on lung adenocarcinoma cell apoptosis and autophagy.

Materials and Methods

Cell culture and transfection

Lung adenocarcinoma cells (A549, NCI-H1975, PC9) and normal human lung cells (BEAS-2B) were purchased from Beijing Northland Biotech. Co., Ltd. (Beijing, China), and maintained in RPMI-1640 medium with penicillin-streptomycin and 10% fetal bovine serum (Invitrogen, Carlsbad, CA, USA). pcDNA vector and pcDNA-BDH2 were acquired from Genepharma (Suzhou, China), and then transfected into A549 cells using Lipofectamine 3000 (Invitrogen).

qRT-PCR

TRIzol method (Invitrogen) was used to extract total RNAs from the cells. cDNAs were then synthesized under Reverse Transcription System (Applied Biosystems, Carlsbad, CA, USA). SYBR Green Master (Roche, Mannheim, Germany) under Applied Bio systems 7500 System was performed for the qRT-PCR analysis of BDH2. Specific primers: BDH2 (Forward: 5'-TTCCAGCGTCAAAGGAGT-3' and Reverse: 5'-TTCCTGGGCACACACAGT-3') and GAPDH (Forward: 5'-GAAGGTGAAGGTCGGAGTC-3' and Reverse: 5'-GAAGATGGTGATGGGATTTC-3') were used in this study.

CCK8 and colony formation assays

A549 with pcDNA transfections were seeded in 96-well plates for 24, 48, 72 or 96 hours. CCK8 solution (Beyotime, Beijing, China) was added and incubated for another 4 hours. Absorbance at 450 nm was measured under microplate reader (Sigma-Aldrich, St. Louis, MO, USA). For colony formation assay, A549 with pcDNA transfections were seeded in a 6-well plate and cultured in the RPMI-1640 medium for 14 days. Cells were fixed and stained with crystal violet before photograph under light microscope (Olympus, Tokyo, Japan).

Flow cytometry

A549 with pcDNA transfections were harvested by trypsin treatment. Cells were then resuspended in binding buffer from Annexin V-FITC Apoptosis Staining/Detection Kit (Abcam, Cambridge, UK). Propidium iodide (PI) and annexin V-FITC were then added into the cell suspension before analysis under FACSCalibur flow cytometer (BD Biosciences, San Jose, CA, USA).

Mouse xenograft assay

All studies involving animals were approved by the Ethics Committee of Sinopharm Han Jiang Hospital and in accordance with the National Institutes of Health Laboratory Animal Care and Use Guidelines. Ten 6–8-week-old female BALB/c nude mice (20–25 g weight) were obtained from ARS/Sprague Dawley Division (Madison, WI, USA), and separated into two groups: pcDNA (n = 5) and pcDNA-BDH2 (n = 5). A549 cells with stable over-expression of BDH2 *via* pLKO-pcDNA-BDH2 or the negative control *via* pLKO-pcDNA were subcutaneously injected in the right flank of nude mice. Tumor volume was calculated every week. Five weeks later, the mice were sacrificed and the tumor tissues were isolated and weighted.

Immunohistochemistry

Tumor tissues were isolated from mice, and fixed in 10% formalin. The tissues were then embedded in paraffin,

and sliced into 4 μm thick sections. After dewaxing and rehydration, the sections were incubated in 3% H_2O_2, immersed in Tris-EDTA buffer containing 0.05% Tween 20, and blocked in 4% dry milk and 0.3% goat serum. The sections were incubated with anti-Ki-67 (1:100; Abcam) antibody, and then incubated with HRP goat anti-rabbit IgG secondary antibody before measurement under a light microscope (Olympus).

Western blot

Cells were lysed in RIPA lysis buffer (Beyotime), and the protein concentration of lysates was determined by Micro BCA protein kit (Pierce, Rockford, IL, USA). Protein samples were separated by 10% SDS-PAGE, and then transferred onto nitrocellulose membranes. Membranes were blocked with 5% defatted milk, and probed with specific antibodies: anti-BDH2 and anti-GAPDH (1:2000, Abcam), anti-Bax and anti-cleaved caspase-3 (1:2500, Abcam), anti-Beclin-1, anti-p62 and anti-LC3 (1:3000, Abcam), anti-AKT and anti-p-AKT (1:3500, Abcam), anti-mTOR and anti-p-mTOR (1:4000, Abcam). Following incubation with horseradish peroxidase-conjugated secondary antibody (1:4500, Abcam) and peroxidase substrate (tetramethylbenzidine), the protein bands were visualized using chemiluminescence (Sigma-Aldrich, St. Louis, MO, USA).

Statistical analysis

All the data with at least triple replicates were expressed as mean ± SEM, and analyzed by Student's *t* test or one-way analysis of variance (ANOVA) under SPSS software. A p value of <0.05 was considered as statistically significant.

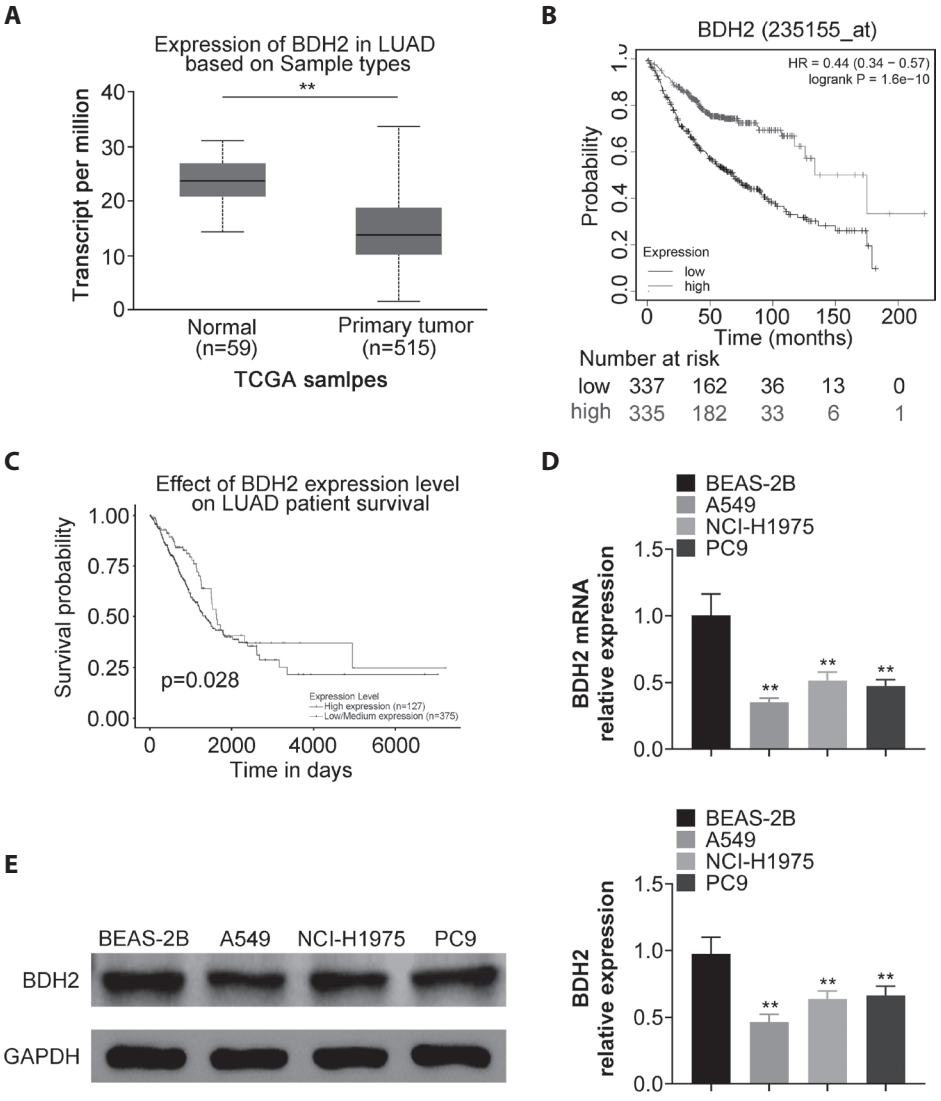

Figure 1. BDH2 was reduced in lung adenocarcinoma. **A.** BDH2 was down-regulated in primary lung adenocarcinoma tissues (n = 515) compared to the normal tissues (n = 59) based on TCGA database. **B.** Ualcan (http://ualcan.path.uab.edu/) analysis showed that patients with high BDH2 expression in lung adenocarcinoma showed higher survival rates than the patients with low BDH2 expression. **C.** Kaplan-Meier Plotter (https://kmplot.com/analysis/) analysis showed that patients with high BDH2 expression in lung adenocarcinoma showed higher survival rates than the patients with low BDH2 expression. **D.** mRNA expression of BDH2 was decreased in lung adenocarcinoma cells (A549, NCI-H1975, PC9) compared to normal human lung cells (BEAS-2B). **E.** Protein expression of BDH2 was decreased in lung adenocarcinoma cells (A549, NCI-H1975, PC9) compared to normal human lung cells (BEAS-2B). ** $p < 0.01$ *vs.* BEAS-2B.

Results

BDH2 was reduced in lung adenocarcinoma

BDH2 was found to be down-regulated in primary lung adenocarcinoma tissues (n = 515) compared to the normal tissues (n = 59) based on TCGA database (Fig. 1A). Data from ualcan (http://ualcan.path.uab.edu/) (Fig. 1B) and Kaplan-Meier Plotter (https://kmplot.com/analysis/) (Fig. 1C) analysis showed that patients with high BDH2 expression in lung adenocarcinoma showed higher survival rates than the patients with low BDH2 expression. Expression of BDH2 was also decreased in lung adenocarcinoma cells (A549, NCI-H1975, PC9) compared to normal human lung cells (BEAS-2B) (Fig. 1D,E). A549 demonstrated the lowest expression of BDH2 among the three lung adenocarcinoma cells, and was used for the subsequently gain-of functional assays.

BDH2 suppressed cell proliferation of lung adenocarcinoma

A549 was transfected with pcDNA-BDH2 for the ectopical expression (Fig. 2A). Ectopical expression of BDH2 reduced cell viability of A549 (Fig. 2B), and decreased cell colonies of A549 (Fig. 2C), suggesting the anti-proliferative effect of BDH2 on lung adenocarcinoma.

BDH2 promoted cell apoptosis of lung adenocarcinoma

Cell apoptosis of A549 was promoted by over-expression of BDH2 (Fig. 3A). Transfection with pcDNA-BDH2 increased protein expression of Bax and cleaved caspase-3 in A549 (Fig. 3B), demonstrating pro-apoptotic role of BDH2 on lung adenocarcinoma.

BDH2 promoted autophagy of lung adenocarcinoma

Protein expression of p62 in A549 was down-regulated by transfection with pcDNA-BDH2 (Fig. 4). Over-expression of BDH2 increased protein expression of LC3 and Beclin-1 in A549, revealing pro-autophagic role of BDH2 on lung adenocarcinoma.

BDH2 suppressed activation of AKT/mTOR of lung adenocarcinoma

Ectopical expression of BDH2 did not affect protein expression of AKT and mTOR in A549 (Fig. 5A). Phosphorylated AKT and mTOR were reduced in A549 cells with transfection of pcDNA-BDH2 (Fig. 5A), indicating that BDH2 suppressed activation of AKT/mTOR signaling in lung adenocarcinoma. Moreover, A549 post pcDNA-BDH2 transfection was incubated with 1 μg/ml SC-79 for 2 h. Inhibition of AKT by SC-79 attenuated BDH2 over-expression-induced decrease of cell viability (Fig. 5B) and increase of cell apoptosis (Fig. 5C) in A549. Moreover, the decreased p62, increased LC3 and Beclin-1 in A549 driven by BDH2 over-expression were also reversed by SC-79 incubation (Fig. 5D). These results showed that BDH2 promoted cell apoptosis and autophagy of lung adenocarcinoma through inactivation of AKT/mTOR.

BDH2 suppressed in vivo lung adenocarcinoma growth

The clinical application of BDH2 on lung adenocarcinoma was investigated by mouse xenograft assay. A549 with stably over-expression of BDH2 was inoculated into the nude mice. Over-expression of BDH2 reduced tumor volume (Fig. 6A) and weight (Fig. 6B) to inhibit the tumor growth.

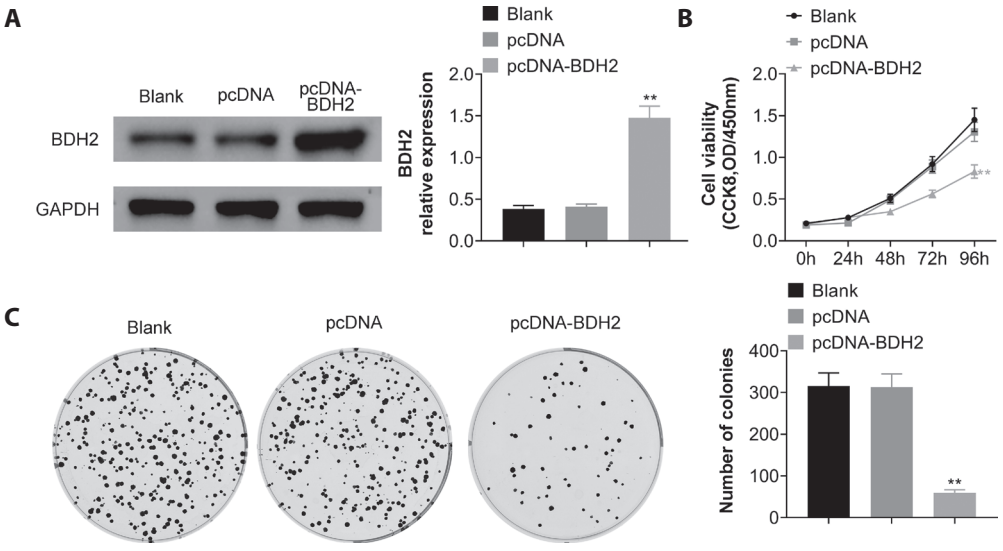

Figure 2. BDH2 suppressed cell proliferation of lung adenocarcinoma. **A.** Transfection with pcDNA-BDH2 increased protein expression of BDH2 in A549. **B.** Ectopical expression of BDH2 reduced cell viability of A549. **C.** Ectopical expression of BDH2 decreased cell colonies of A549. ** $p < 0.01$ *vs.* pcDNA.

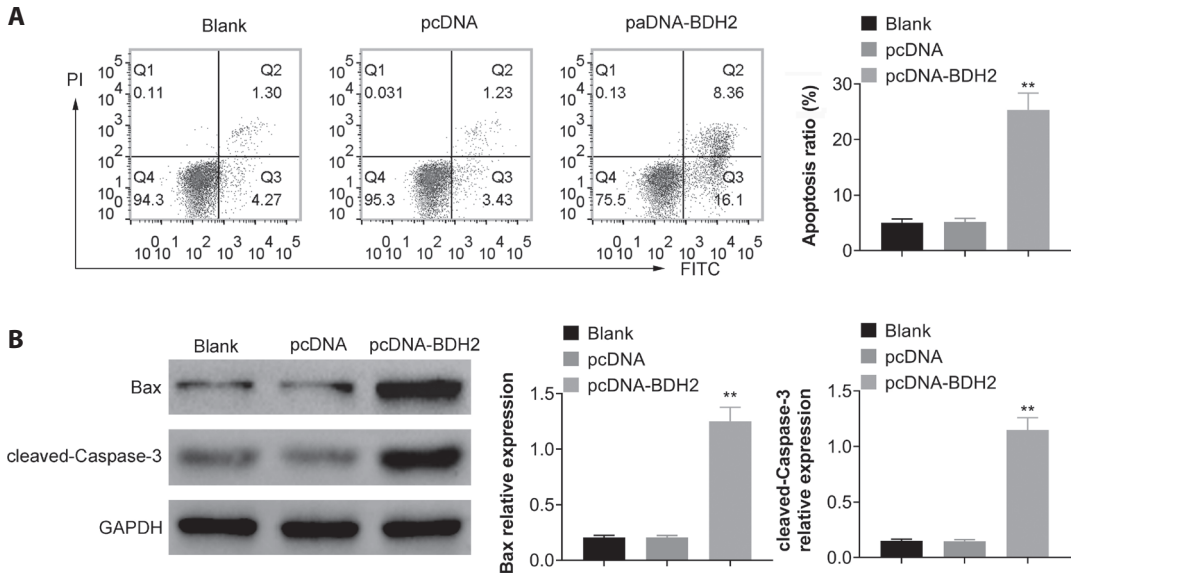

Figure 3. BDH2 promoted cell apoptosis of lung adenocarcinoma. **A.** Ectopical expression of BDH2 promoted cell apoptosis of A549. **B.** Ectopical expression of BDH2 increased protein expression of Bax and cleaved caspase-3 in A549. ** $p < 0.01$ *vs.* pcDNA.

Immunohistochemical staining indicated that the expression of Ki67 was reduced in the xenograft tumor tissues injected with pcDNA-BDH2 (Fig. 6C). Over-expression of BDH2 increased protein expression of Bax and LC3 (Fig. 6D), suggesting that BDH2 suppressed xenograft tumor growth of lung adenocarcinoma through promotion of cell apoptosis and autophagy.

Discussion

Previous study has shown that iron regulation provides energy for cytokine production, membrane rigidity, cell proliferation, and growth, thus participating in pathophysiological conditions, including cancers (Cronin et al. 2019). Efflux, storage, and iron uptake were disturbed in lung cancer, and reprogramming of iron metabolism is considered as potential tools for prognosis and therapy of lung cancer (Xiong et al. 2014). Iron metabolism has also been reported to be involved in lung adenocarcinoma progression (Thielmann et al. 2019; Yao et al. 2021). Since BDH2 has been shown to regulate iron retention to retard nasopharyngeal carcinoma cell proliferation and metastasis (Li et al. 2020), the effect of BDH2 on lung adenocarcinoma progression was investigated in this study.

Expression of BDH2 was found to be down-regulated in lung adenocarcinoma tissues and cells. High BDH2 expression was reported to be associated with low complete response rate and short overall survival of patients with acute myeloid leukemia (Yang et al. 2013). Whether BDH2 indicated poor prognosis of patients with lung adenocarcinoma or exerted diagnostic role on lung adenocarcinoma should be investigated in further research *via* determination of relation between BDH2 expression

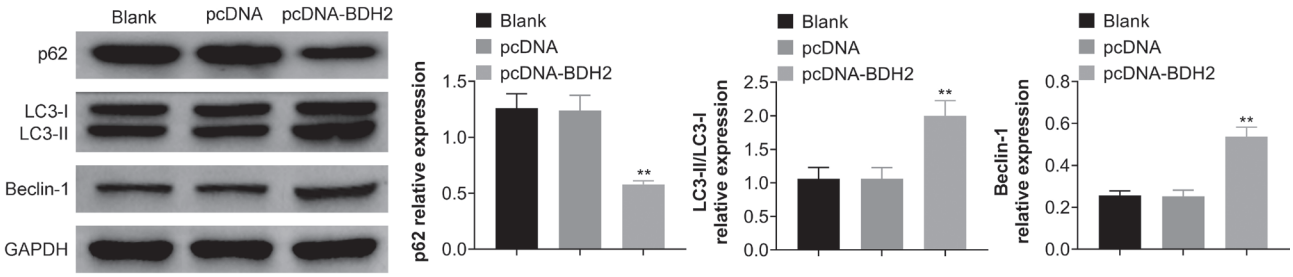

Figure 4. BDH2 promoted autophagy of lung adenocarcinoma. Ectopical expression of BDH2 reduced protein expression of p62 in A549, while increased protein expression of LC3 and Beclin-1. ** $p < 0.01$ *vs.* pcDNA.

and clinicopathological variables of lung adenocarcinoma patients.

Functional assays showed that BDH2 contributed to the suppression of lung adenocarcinoma cell proliferation. BDH2 functioned as an anti-apoptosis factor in acute myeloid leukemia through caspase-3-independent pathway (Yang et al. 2013). Knockdown of BDH2 also promoted cell apoptosis of esophageal squamous cell carcinoma through caspase-3-dependent apoptotic pathway (Zang et al. 2016). However, BDH2 promoted cell apoptosis of hepatocellular carcinoma (Liang et al. 2019) and gastric cancer (Liu et al. 2020) through decrease of Bcl-2, increase of Bax and cleaved caspase-3. In this study, over-expression of BDH2 promoted protein expression of Bax and cleaved caspase-3 in lung adenocarcinoma cells to induce cell apoptosis, suggesting that BDH2 functioned as a pro-apoptotic factor in lung adenocarcinoma *via* caspase-3-dependent apoptotic pathway. However, the effect of BDH2 on cell migration and invasion of lung adenocarcinoma should be investigated in further research.

Interaction between cell apoptosis and autophagy contributes to anti-tumor therapy of lung adenocarcinoma (Han et al. 2014). Autophagic biomarkers, such as Beclin-1, LC3, p62, were also implicated in the tumor cell apoptosis (Xie et al. 2020). BDH2 has been reported to suppress autophagy of hepatocellular carcinoma through the unfolded protein response (Liang et al. 2019), while induced autophagy of gastric cancer through Nrf2-mediated secretion

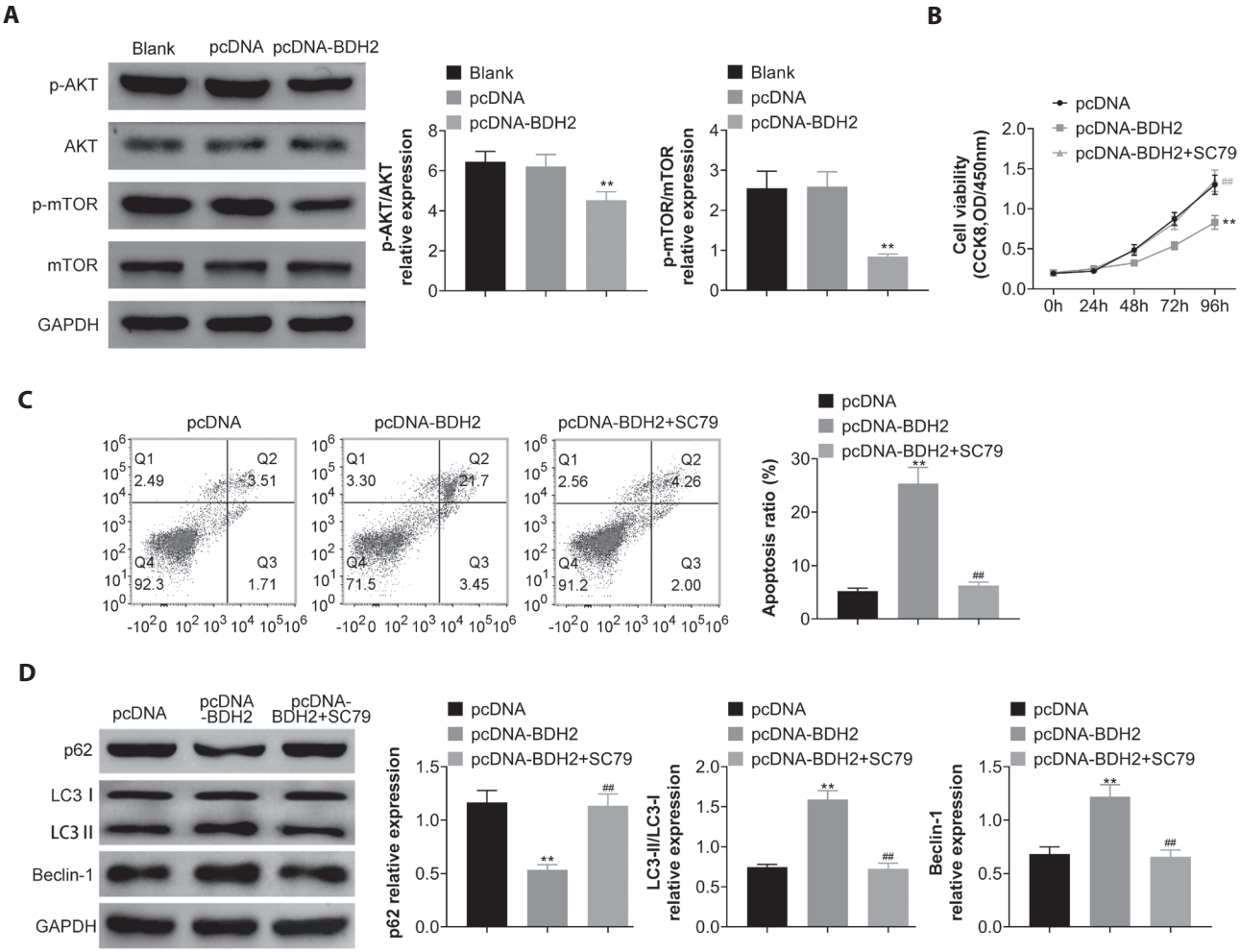

Figure 5. BDH2 suppressed activation of AKT/mTOR of lung adenocarcinoma. **A.** Ectopical expression of BDH2 reduced phosphorylated AKT and mTOR levels in A549. **B.** Inhibition of AKT by SC-79 attenuated BDH2 over-expression-induced decrease of cell viability in A549. **C.** Inhibition of AKT by SC-79 attenuated BDH2 over-expression-induced increase of cell apoptosis in A549. **D.** Inhibition of AKT by SC-79 attenuated BDH2 over-expression-induced decrease of p62, increase of LC3 and Beclin-1 in A549. ** $p < 0.01$ *vs.* pcDNA; ## $p < 0.01$ *vs.* pcDNA-BDH2.

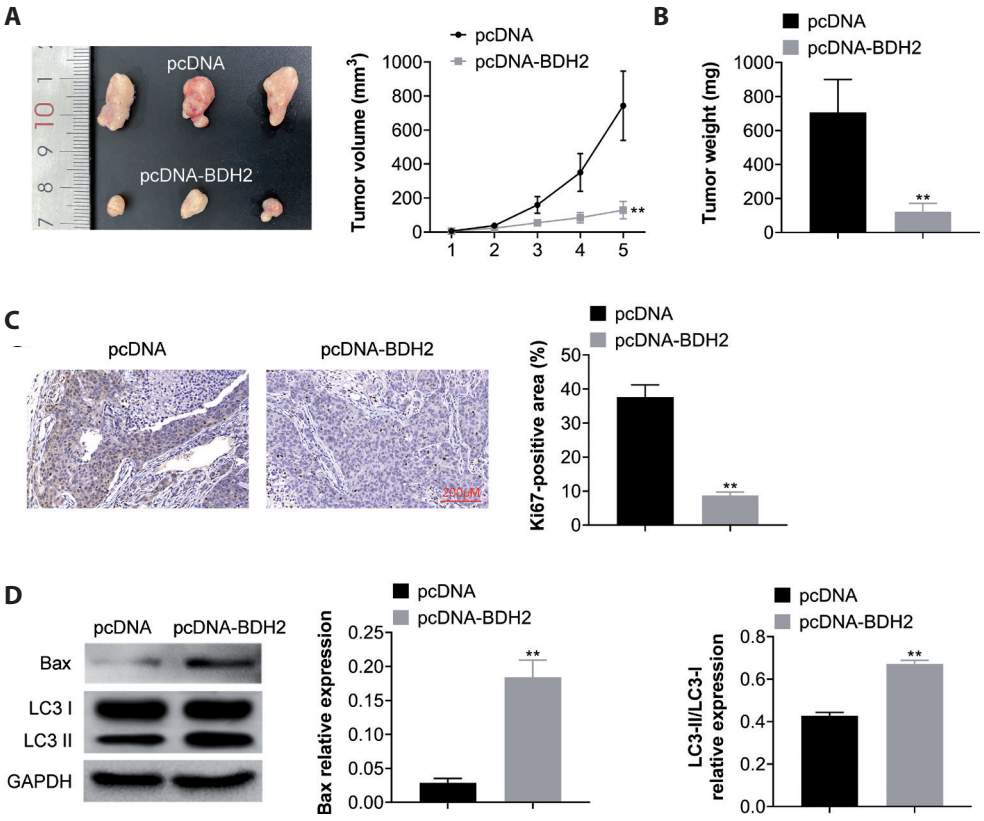

Figure 6. BDH2 suppressed *in vivo* lung adenocarcinoma growth. **A.** Over-expression of BDH2 reduced tumor volume. **B.** Over-expression of BDH2 reduced tumor weight. **C.** Over-expression of BDH2 reduced the expression of Ki67. **D.** Over-expression of BDH2 reduced increased protein expression of Bax and LC3. ** $p < 0.01$ *vs.* pcDNA.

of reactive oxygen species (Liu et al. 2020). Here, protein expression of p62 was reduced in lung adenocarcinoma cells by over-expression of BDH2, LC3 and Beclin-1 were enhanced by over-expression of BDH2, indicating that BDH2 promoted autophagy of lung adenocarcinoma to suppress tumor progression. Emerging evidence has shown that AKT pathway is implicated in the cellular processes, including apoptosis, autophagy, redox states, and metabolism (Xie et al. 2022). Blockade of AKT/mTOR signaling participated in antrodin C-mediated crosstalk between apoptosis and autophagy of lung adenocarcinoma (Yang et al. 2019). Cabazitaxel decreased phosphorylation of Akt and mTOR in lung adenocarcinoma cells and promoted autophagy and cell death (Huo et al. 2016). Moreover, BDH2 has been shown to suppress phosphorylation of AKT and mTOR to induce cell apoptosis and autophagy of gastric cancer (Liu et al. 2020). Results in this study demonstrated that p-AKT and p-mTOR in lung adenocarcinoma cells were reduced by over-expression of BDH2, indicating that BDH2 contributed to the apoptosis and autophagy of lung adenocarcinoma through inactivation of AKT/mTOR signaling.

In conclusion, this study identified a novel tumor suppressor, BDH2, in lung adenocarcinoma. BDH2 played the tumour-suppressive role through promotion of AKT/mTOR-mediated apoptosis and autophagy. These findings revealed a potential target for the treatment of lung adenocarcinoma. However, the *in vivo* suppressive role of BDH2 on lung adenocarcinoma should be investigated in further research.

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Competing interests. The authors state that there are no conflicts of interest to disclose.

Ethics approval. Ethical approval was obtained from the Ethics Committee of Sinopharm Han Jiang Hospital.

Contribution of authors. YN and XM designed the study, supervised the data collection; FC analyzed the data, interpreted the data; XZ prepare the manuscript for publication and reviewed the draft of the manuscript. All authors have read and approved the manuscript.

References

Barone M, Di Nuzzo D, Cipollone G, Camplese P, Mucilli F (2015): Oligometastatic non-small cell lung cancer (NSCLC): adrenal metastases. Experience in a single institution. Updates Surg. **67,** 383-387

https://doi.org/10.1007/s13304-015-0336-x
Chen C, Gao H, Su X (2021): Autophagy-related signaling pathways are involved in cancer (Review). Exp. Ther. Med. **22,** 710-710 https://doi.org/10.3892/etm.2021.10142
Cronin SJF, Woolf CJ, Weiss G, Penninger JM (2019): The role of iron regulation in immunometabolism and immune-related disease. Front. Mol. Biosci. **6,** 116 https://doi.org/10.3389/fmolb.2019.00116
Fu Y, Liu F, Cao S, Zhang J, Wang H, Wu B, Song Y, Duo S, Li X, Bao S (2021): Bdh2 deficiency promotes endoderm-biased early differentiation of mouse embryonic stem cells. Front. Cell. Dev. Biol. **9,** 655145-655145 https://doi.org/10.3389/fcell.2021.655145
Gardiner N, Jogai S, Wallis A (2014): The revised lung adenocarcinoma classification-an imaging guide. J. Thorac. Dis. **6,** S537-S546
Han R, Liang H, Qin ZH, Liu CY (2014): Crotoxin induces apoptosis and autophagy in human lung carcinoma cells in vitro via activation of the p38MAPK signaling pathway. Acta Pharmacol. Sin. **35,** 1323-1332 https://doi.org/10.1038/aps.2014.62
Huo R, Wang L, Liu P, Zhao Y, Zhang C, Bai B, Liu X, Shi C, Wei S, Zhang H (2016): Cabazitaxel-induced autophagy via the PI3K/Akt/mTOR pathway contributes to A549 cell death. Mol. Med. Rep. **14,** 3013-3020 https://doi.org/10.3892/mmr.2016.5648
Kudo F, Watanabe Y, Iwai Y, Miwa C, Nagai Y, Ota H, Yabe H, Demitsu T, Hagiwara K, Koyama N, Koyama S (2018): Advanced lung adenocarcinoma with nivolumab-associated dermatomyositis. Intern. Med. **57,** 2217-2221 https://doi.org/10.2169/internalmedicine.9381-17
Li B, Liao Z, Mo Y, Zhao W, Zhou X, Xiao X, Cui W, Feng G, Zhong S, Liang Y, et al. (2020): Inactivation of 3-hydroxybutyrate dehydrogenase type 2 promotes proliferation and metastasis of nasopharyngeal carcinoma by iron retention. Br. J. Cancer **122,** 102-110 https://doi.org/10.1038/s41416-019-0638-8
Liang H, Xiong Z, Li R, Hu K, Cao M, Yang J, Zhong Z, Jia C, Yao Z, Deng M (2019): BDH2 is downregulated in hepatocellular carcinoma and acts as a tumor suppressor regulating cell apoptosis and autophagy. J. Cancer **10,** 3735-3745 https://doi.org/10.7150/jca.32022
Liang H, Xiong Z, Yao Z, Li R, Kong W, Xu J, Cao M-z, Deng M (2018): Inhibiting effect and its mechanism of BDH2 gene on the proliferation of liver cancer cells. Chin. J. Hepat. Surg. **7,** 327-331
Liu JZ, Hu YL, Feng Y, Jiang Y, Guo YB, Liu YF, Chen X, Yang JL, Chen YY, Mao QS, Xue WJ (2020): BDH2 triggers ROS-induced cell death and autophagy by promoting Nrf2 ubiquitination in gastric cancer. J. Exp. Clin. Cancer Res. **39,** 123-123 https://doi.org/10.1186/s13046-020-01620-z
Milošević B, Pejić D, Momčičević D, Kovačević P, Stanetić M, Dragić S (2016): Quality of life in lung cancer patients due to treatment. Signa Vitae **11,** 47-50 https://doi.org/10.22514/SV112.062016.10
Tian R, Zhang C, Xiong F, Chen H (2020): PCAT1/miR-129/ABCB1 axis confers chemoresistance in non-small cell lung cancer. Front. Biosci. (Landmark) **25,** 948-960 https://doi.org/10.2741/4842
Thielmann CM, Costa da Silva M, Muley T, Meister M, Herpel E, Muckenthaler MU (2019): Iron accumulation in tumor-associated macrophages marks an improved overall survival in patients with lung adenocarcinoma. Sci. Rep. **9,** 11326-11326 https://doi.org/10.1038/s41598-019-47833-x
Wan Q, Du Z, Fang Z, Cheng H, Li C, Zhou X (2020): Matrine induces apoptosis and autophagy in human lung adenocarcinoma cells via upregulation of Cavin3 and suppression of PI3K/AKT pathway. J. BUON **25,** 1512-1516
Wu L, Wen Z, Song Y, Wang L (2021): A novel autophagy-related lncRNA survival model for lung adenocarcinoma. J. Cell. Mol. Med. **25,** 5681-5690 https://doi.org/10.1111/jcmm.16582
Xie X, Shu R, Yu C, Fu Z, Li Z (2022): Mammalian AKT, the emerging roles on mitochondrial function in diseases. Aging Dis. **13,** 157-174 https://doi.org/10.14336/AD.2021.0729
Xie Q, Liu Y, Li X (2020): The interaction mechanism between autophagy and apoptosis in colon cancer. Transl. Oncol. **13,** 100871-100871 https://doi.org/10.1016/j.tranon.2020.100871
Xiong W, Wang L, Yu F (2014): Regulation of cellular iron metabolism and its implications in lung cancer progression. Med. Oncol. **31,** 28 https://doi.org/10.1007/s12032-014-0028-2
Yang H, Bai X, Zhang H, Zhang J, Wu Y, Tang C, Liu Y, Yang Y, Liu Z, Jia W, Wang W (2019): Antrodin C, an NADPH dependent metabolism, encourages crosstalk between autophagy and apoptosis in lung carcinoma cells by use of an AMPK inhibition-independent blockade of the Akt/mTOR pathway. Molecules **24,** 993 https://doi.org/10.3390/molecules24050993
Yang WC, Tsai WC, Lin PM, Yang MY, Liu YC, Chang CS, Yu WH, Lin SF (2013): Human BDH2, an anti-apoptosis factor, is a novel poor prognostic factor for de novo cytogenetically normal acute myeloid leukemia. J. Biomed. Sci. **20,** 58-58 https://doi.org/10.1186/1423-0127-20-58
Yao J, Chen X, Liu X, Li R, Zhou X, Qu Y (2021): Characterization of a ferroptosis and iron-metabolism related lncRNA signature in lung adenocarcinoma. Cancer Cell. Int. **21,** 340 https://doi.org/10.1186/s12935-021-02027-2
Zang W, Wang T, Wang Y, Chen X, Du Y, Sun Q, Li M, Dong Z, Zhao G (2016): Knockdown of long non-coding RNA TP73-AS1 inhibits cell proliferation and induces apoptosis in esophageal squamous cell carcinoma. Oncotarget **7,** 19960-19974 https://doi.org/10.18632/oncotarget.6963

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